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Phloretin affects the fast potassium channels in frog nerve fibres

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Abstract. The effect of phloretin (20–100 μ*M*), a dipolar organic compound, on the voltage clamp currents of the frog node of Ranvier has been investigated. The Na currents are simply reduced in size but not otherwise affected. Phloretin has no effect on the slow 4-aminopyridine-resistant K channels. However, the voltage dependence and time course of the fast K conductance (g_K) is markedly altered. The g_K (E) curve, determined by measuring fast tail currents at different pulse potentials, normally exhibits a bend at −50 mV, indicating the existence of two tpyes of fast K channels. Phloretin shifts the $g_K(E)$ curve to more positive potentials, reduces its slope and its maximum and abolishes the distinction between the two tpyes of fast K channels. The effect becomes more pronounced with time. Phloretin also markedly slows the opening of the fast K channels, but has much less effect on the closing. Opening can be accelerated again by a long depolarizing prepulse which presumably removes part of the phloretin block. It is concluded that phloretin selectively affects the fast K channels of the nodal membrane. The results are compared with similar observations on the squid giant axon.

Key words: Nerve fibre – Potassium channel – Voltage clamp – Phloretin

Introduction

Phloretin, the aglycone of phlorhizin, is a polar substance with a dipole moment of 5.6 D. Long known for its inhibitory effect on glucose resorption ("phlorhizin diabetes"), phloretin also markedly affects ion channels in artificial lipid bilayers (Andersen et al., 1976) and squid giant axons (Strichartz et al. 1980; Spires and Begenisich 1989). In lipid bilayers treated with ion carrier or lipophilic ions phloretin dramatically increases cation conductances and decreases anion conductances. In squid giant axons, phloretin (10–50 μ*M*) has several effects on K channels: it reduces the K channel conductance g_K, shifts the voltage dependence of g_K by +30 to 40 mV, decreases its slope and slows the opening of the K channels. Na channels are less sensitive: phloretin (60 μ*M*) shifts the voltage dependence of g_{Na} by less than +10 mV. Phloretin can be used to identify a component of gating current that is associated with K channels.

The present paper describes the effect of phloretin on the K currents of the frog node of Ranvier. In this preparation, three classes of K channels can be distinguished: fast 1, fast 2 and slow (Dubois 1981). We show that phloretin markedly affects the components fast 1 and fast 2, but not the slow.

Some of the results have been published in abstract form (Klusemann and Meves 1991).

Methods

The experiments were done on single motor or sensory nerve fibres from the tibial or peroneal nerve of the frog *Rana esculenta*. A node of Ranvier was voltage clamped at 12–15 °C by the method of Nonner (1969). The fibre was cut on both sides of the node, at a distance of about 0.75 mm on one side and at about twice that distance on the other side. The ends of the fibre were bathed in 117 m*M* KCl. The potential at which the Na current was 70% of the maximum Na current (measured with a prepulse of −40 mV and 50 ms duration) was taken as the normal resting potential ($E = -70$ mV). The holding potential was −70 or −100 mV. Membrane currents were filtered at 10 kHz and sampled on-line at 10 μs intervals by a 12 bit A/D converter (Hof 1986). To obtain absolute values of membrane current, the recording resistance was assumed to be 10 *M* Ω. To correct for capacitative and leakage current (I_c and I_L) the current associated with a −30 mV pulse was measured (usually by averaging three sweeps), appropriately scaled and subtracted. Unless otherwise stated, the figures show records corrected for I_c and I_L.

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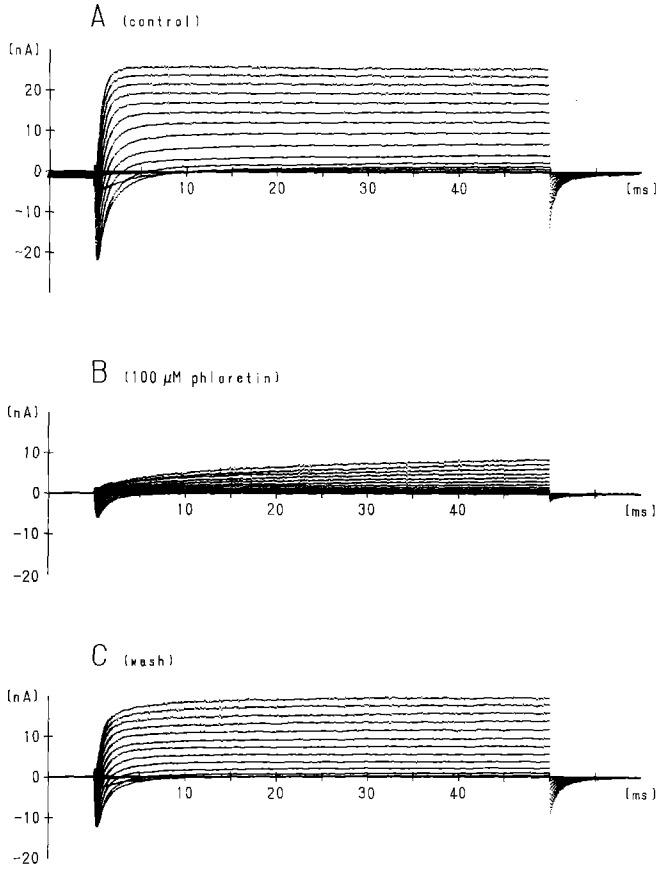


Fig. 1 A–C. Effect of 100 μ*M* phloretin on I_{Na} and I_K in Ringer's solution. **A,** control; **B,** after 5 min in Ringer's solution with 100 μ*M* phloretin; **C,** after 5 min wash. Pulse potential −60 to +80 mV in 10 mV steps. Sensory fibre. Holding potential −70 mV. Temperature 12 °C

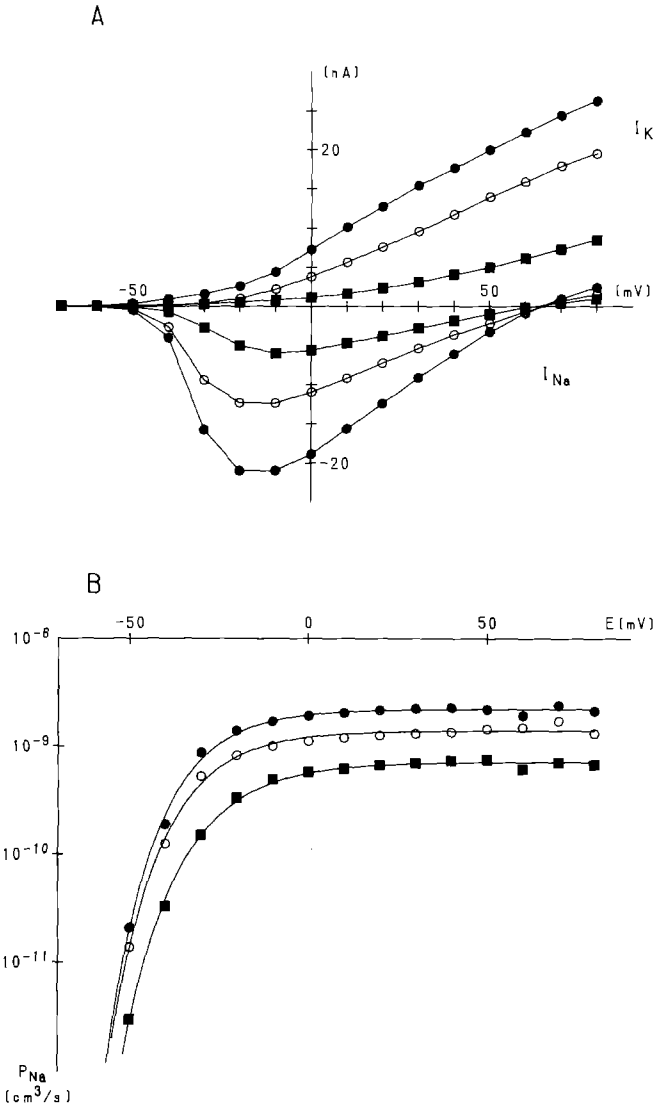


Fig. 2 A and B. Current-voltage curves and permeability-voltage curves from the experiment of Fig. 1. **A** peak I_{Na} and I_K at 50 ms plotted against pulse potential for control (●), 100 μ*M* phloretin (■) and wash (○). **B** peak Na permeability P_{Na} (on a logarithmic scale) plotted against pulse potential; P_{Na} calculated from I_{Na} according to (2) of Dodge and Frankenhaeuser (1959), assuming for the reversal potential of the Na current $E_{rev} = 65$ mV in control and wash and $E_{rev} = 62.5$ mV in 100 μ*M* phloretin

The node was superfused with Ringer's solution (110 m*M* NaCl, 2.5 m*M* KCl, 1.8 m*M* $CaCl_2$) or isotonic KCl solution (117 m*M* KCl, 1.8 m*M* $CaCl_2$). Both solutions contained 4 m*M* morpholinopropane sulfonic acid (MOPS); their pH was adjusted to 7.2 with 1 N NaOH or KOH, respectively. The isotonic KCl solution also contained 300 nM tetrodotoxin to block the Na channels. Phloretin was dissolved in dimethylsulfoxide (DMSO). The DMSO concentration in the final solution was in most experiments 0.05–0.1% and never exceeded 0.2%.

Results

Experiments in Ringer's solution

Figure 1 shows the typical effect of phloretin on the Na and K currents of the node of Ranvier: a drastic, but partly reversible reduction of I_{Na} and I_K and a marked slowing in the turning-on of I_K. The current-voltage curves for I_{Na} at peak and I_K at 50 ms are plotted in Fig. 2 A. It can be seen that the Na and K currents in phloretin (■) are only 20–30% of the control currents (●). The reversal potential of the Na current changes little. Converting peak Na current I_{Na} into peak Na permeability P_{Na} yields the $P_{Na}(E)$ curves in Fig. 2 B. The maximum P_{Na} values reached at $E > 50$ mV are $2.1 \cdot 10^{-9}$ cm³/s in control (●) and $0.7 \cdot 10^{-9}$ cm³/s in phloretin (■), again a decrease to 33%. In addition, the $P_{Na}(E)$ curve in phloretin is slightly shifted to the right compared with control. A fifth of the maximum P_{Na} is reached at −37 mV in control (●) and at −31 mV in phloretin (■). At least half of the +6 mV shift can be accounted for by the 220 kΩ resistance in series with the nodal membrane (Drouin and Neumcke 1974). There was also a 15 mV shift of the curve relating time to peak to pulse potential, part of it is also a series resistance artifact.

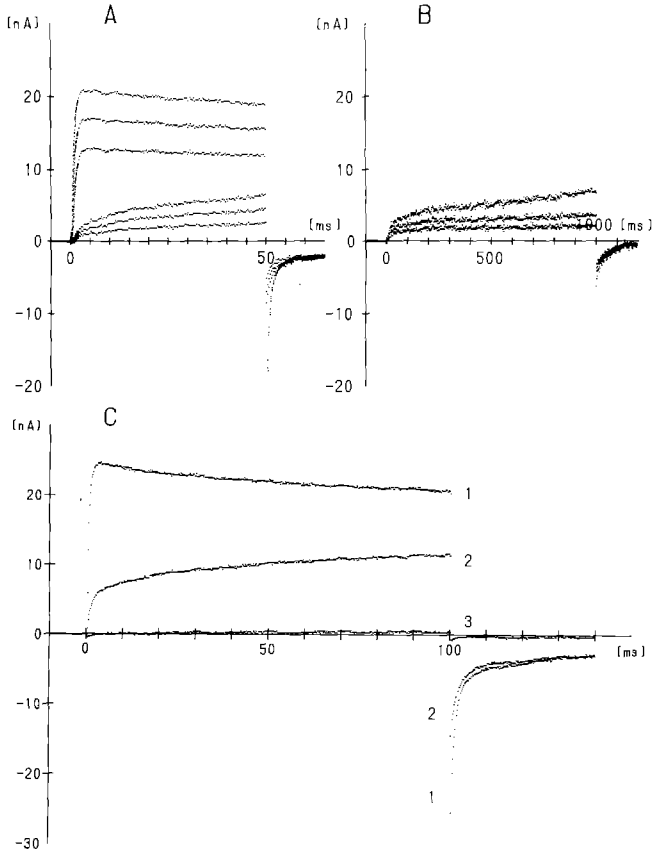


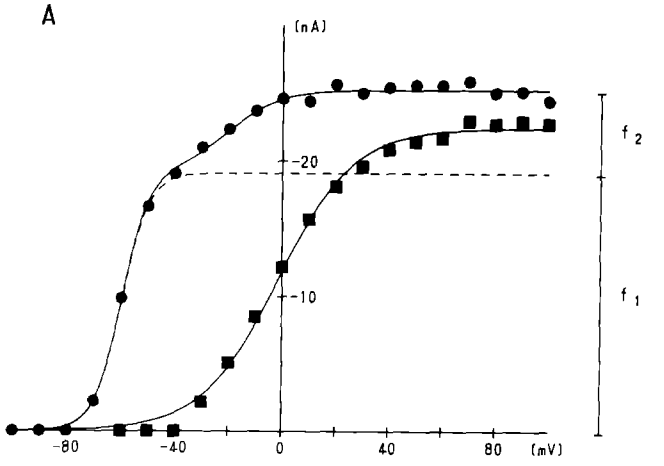
Fig. 3 A–C. Effect of 40 μ*M* phloretin and 12 m*M* TEA on I_K in isotonic KCl solution. **A** currents during 50 ms pulses from holding potential (−100 mV) to 60, 80 and 100 mV in control (larger currents) and after 10 min in 40 μ*M* phloretin (smaller currents). **B** currents during 1 s pulses to 60, 80 and 100 mV after 40 min in 40 μ*M* phloretin. **C** Currents during and after 100 ms pulses from holding potential (−100 mV) to 100 mV in control (record 1), 40 μ*M* phloretin (record 2) and 40 μ*M* phloretin +12 m*M* TEA (record 3). Two different sensory fibres in A, B and in C. Temperature 12 °C

Experiments in isotonic KCl solution

In order to minimize effects of perinodal K accumulation, all further experiments (Fig. 3–9) were done in isotonic KCl solution. Figure 3 shows an experiment with 50 ms (A) and 1 s (B) pulses. Phloretin (40 μ*M*) markedly reduces I_K and slows its turning-on. The K outward currents in control (larger currents in A) decay slowly during the 50 ms pulses. By contrast, the currents in 40 μ*M* phloretin (smaller currents in A) rise slowly. The currents continue to rise during the entire duration of the 1 s pulses (B).

The slowly rising outward current in 40 μ*M* phloretin is blocked by 12 m*M* TEA (Fig. 3C), indicating that it is a current through the K channels and not a leakage current. Figure 3C also illustrates the tail currents which occur at the pulse end upon repolarization to the holding potential. Normally, the tail current consists of a fast and a slow component (record 1). Phloretin reduces the fast but not the slow component (record 2) whereas 12 m*M* TEA block both components almost completely (record 3).

Quantitative information about the effect of phloretin on the fast component of the tail current is given in Fig. 4. The tail currents (recorded at the holding potential) were fitted with the sum of two exponentials and the $t=0$ values of the fast component were plotted against pulse potential. The resulting curves (● in Fig. 4A and B) indicate two components (Dubois 1981). The relatively large size of the first component in Fig. 4A is typical for a motor fibre. The large second component in Fig. 4B is characteristic for a sensory fibre. A short application of 40 μ*M* phloretin shifts the curve to more positive potentials, reduces its slope and its maximum and abolishes the distinction between the two components (Fig. 4A). The effect becomes more and more pronounced, the longer phloretin is applied (Fig. 4B). Neither the slow compo-



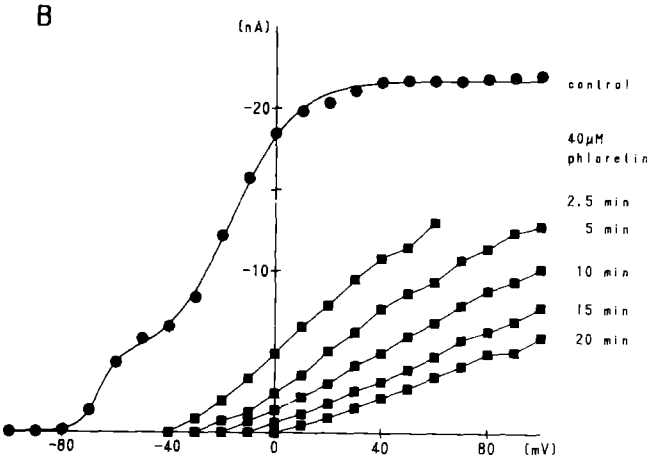


Fig. 4 A and B. Fast component of tail current versus pulse potential. ●, control in isotonic KCl solution. ■, isotonic KCl with 40 μ*M* phloretin, **A** motor fibre; measurements begin after 5 min application of 40 μ*M* phloretin. **B** sensory fibre, same fibre as in Fig. 3C; measurements at different times in 40 μ*M* phloretin. Temperature 12 °C in **A** and **B**. Points ● in **A** and **B** and points ■ in **A** were fitted by the equation

$$I_{fast} = \max I_1/(1+\exp((E_{mid1}-E)/k_1)) + \max I_2/(1+\exp((E_{mid2}-E)/k_2)) \quad (1)$$

with the following parameters:

	A, points ●	B, points ●	A, points ■
max I_1	−19.2 nA	− 4.5 nA	−22.4 nA
max I_2	− 6.0 nA	−17.2 nA	0
E_{mid1}	−60.1 mV	−66.2 mV	− 1.5 mV
E_{mid2}	−20.2 mV	−16.8 mV	–
k_1	5.0 mV	3.6 mV	14.1 mV
k_2	9.4 mV	12.0 mV	–

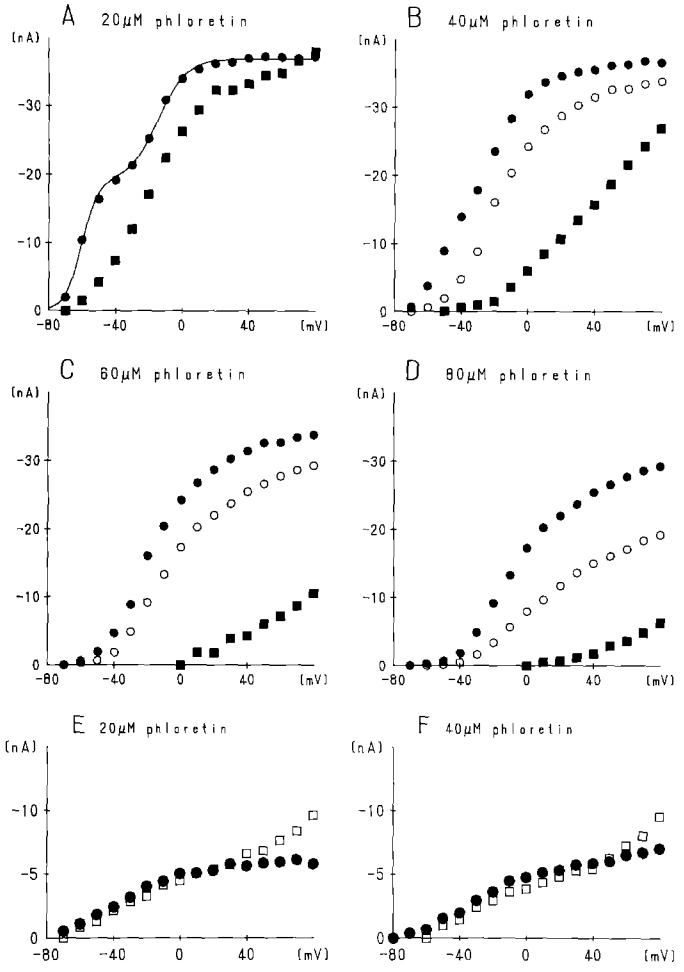


Fig. 5A–F. Fast and slow component of tail current versus pulse potential for different phloretin concentrations. **A–D** fast component. **A** control (●) and 20 μ*M* phloretin (■); **B** control (●), 40 μ*M* phloretin (■) and wash (○); **C** and **D** same for 60 and 80 μ*M* phloretin. **E–F** slow component. **E** control (●) and 20 μ*M* phloretin (□); **F** control (●) and 40 μ*M* phloretin (□). Motor fibre. Holding potential −100 mV. Temperature 12 °C. The control points in A were fitted with (1) (see Fig. 4 legend) with the parameters max $I_1 = -19.1$ nA, max $I_2 = -18.0$ nA, $E_{mid1} = -60.5$ mV, $E_{mid2} = -14.7$ mV, $k_1 = 4.8$ mV, $k_2 = 8.5$ mV

nent nor the time constants of the fast and slow components are altered.

The potential $E_{1/3}$ at which the fast tail current component equals a third of the control maximum served as a quantitative estimate of the curve shift and its continual increase with time. In Fig. 4B, $E_{1/3}$ jumped from −37 to +14 mV in the first 2.5 min of phloretin application and then grew further at a rate of 6.4 mV/min. In a similar experiment on a motor fibre, also with 40 μ*M* phloretin, $E_{1/3}$ was −64 mV in control, +16 mV after 2.5 min in phloretin and subsequently rose at a rate of 7.5 mV/min.

The phloretin effect increases with increasing phloretin concentration. This is demonstrated in Fig. 5. Four different phloretin concentrations (20, 40, 60, 80 μ*M*) were applied. After 5 min application measurements were started. They took 4 min and were followed by a wash with phloretin-free isotonic KCl solution. After 5–6 min wash a new series of measurements began, which again lasted 4 min and was followed by the next higher phloretin concentration. A–D show fast tail current versus pulse potential in phloretin (■) together with the preceding control or wash (●) and the following wash (○) (The latter was omitted in A for clarity). It is clear that the phloretin effect is only partially removed by the washes. The distinction between the two fast components (see fit of points ● in A) vanishes during the first phloretin treatment and does not come back later. The absolute value of the maximum tail current amplitude reached at positive potentials decreases from 37.1 nA in A (symbols ●) to about 20 nA in D (symbols ○), presumably reflecting an increase of membrane-bound phloretin. With increasing phloretin concentration the curves in phloretin (■) become flatter and flatter and are more and more shifted to the right.

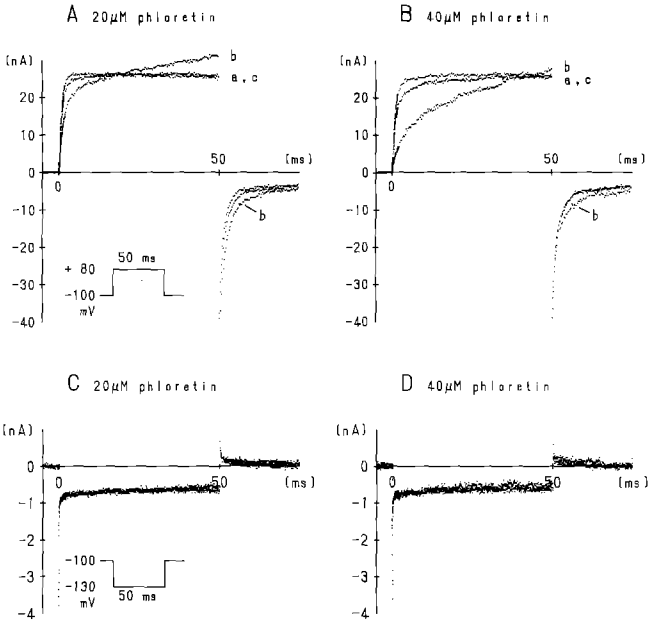


Fig. 6. Supernormal K outward current in 20 μ*M* (**A**) and 40 μ*M* (**B**) phloretin. *Top*: Currents during 50 ms pulses from holding potential (−100 mV) to 80 mV in control (a), phloretin (b) and wash (c). *Bottom*: Currents during 50 ms pulses from holding potential (−100 mV) to −130 mV; records in control and phloretin superimposed. Same experiment as Fig. 5

In the experiment of Fig. 5 we saw a phenomenon which was observed in only 6 out of our 60 experiments: a supernormal K outward current at the end of strong depolarizing pulses in 20 μ*M* phloretin (Fig. 6A). It disappeared upon washing and was not due to an increase of the (linear) leakage (Fig. 6C). There was also an increase of the slow component of the tail current (Fig. 6A) which began at pulse potentials >40 mV (Fig. 5E). An indication of the phenomenon was also seen in 40 μ*M* phloretin (Fig. 6B, Fig. 5F). In some experiments the fast component of the tail current became larger than in the control. A possible explanation of the phenomenon will be mentioned below.

Experiments on DTX- and 4-AP-treated fibres

On frog and *Xenopus* fibres, dendrotoxin (DTX) selectively blocks the first component of the fast tail current (Benoit and Dubois 1986; Bräu et al. 1990). We tested the

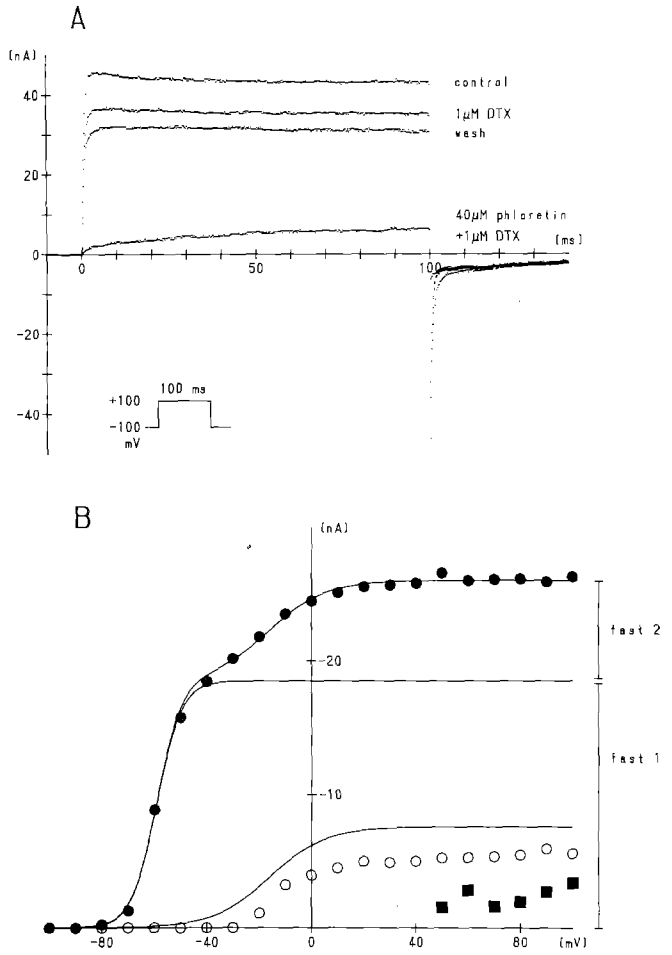


Fig. 7 A and B. Effect of 40 μ*M* phloretin on fibres treated with 1 μ*M* dendrotoxin (DTX) to block the first component of the fast tail current. **A** records from a sensory fibre showing currents associated with a 100 ms pulse from holding potential (−100 mV) to 100 mV in isotonic KCl, isotonic KCl with 1 μ*M* DTX, isotonic KCl with 1 μ*M* DTX and 40 μ*M* phloretin and again isotonic KCl. **B** fast component of tail current versus pulse potential for a motor fibre in isotonic KCl (●), same with 1 μ*M* DTX (○) and with 1 μ*M* DTX and 40 μ*M* phloretin (■). The control points were fitted with (1) (see Fig. 4 legend) with the parameters max $I_1 = -17.6$ nA, max $I_2 = -8.5$ nA, $E_{mid1} = -59.2$ mV, $E_{mid2} = -19.3$ mV, $k_1 = 5.1$ mV, $k_2 = 13.0$ mV. Temperature 12 °C

effect of 40 μ*M* phloretin on fibres treated with 1 μ*M* DTX in order to demonstrate that phloretin affects the second component. Figure 7 shows an experiment on a sensory fibre (A) and an experiment on a motor fibre (B). The second component, which remains largely unaffected by DTX, is relatively large in A and small in B. The remaining second component in A shows the typical phloretin effect: a reversible reduction in size and a slowing of kinetics. In B, the tail current remaining in DTX (○) is only slightly smaller than fast 2 in control (lower curve). Phloretin clearly shifts the points in the positive direction (■).

We also did the reverse experiment and tested 1 μ*M* DTX on fibres treated with 40 μ*M* phloretin. The outward currents which were decreased and slowed by phloretin were further decreased by 1 μ*M* DTX; in two experiments I_K (measured at the end of 100 ms pulses to 100 mV) decreased to 26 and 50% of its size in 40 μ*M* phloretin without DTX.

Treatment with 1 m*M* 4-aminopyridine (4-AP) abolishes the fast tail current but has no effect (Dubois 1981) or less effect (Pappone and Cahalan 1984; Plant 1986) on the slow component. Phloretin did not decrease the slow tail current remaining in 1 m*M* 4-AP; in 5 out of 6 experiments even a slight increase of the slow component in phloretin was observed. The outward current recorded during a depolarizing pulse was markedly decreased by 1 m*M* 4-AP (as in Fig. 6 of Ulbricht and Wagner 1976). The remaining 4-AP-resistant outward current showed considerable run-down (Plant, 1986), but did not appear to be significantly affected by phloretin.

Experiments with prepulses

In a first series of experiments we used double pulses as shown in the inset of Fig. 8. The first and second pulse were of equal size and duration and separated by a 0.5 ms pause. Figure 8 shows the currents produced by the second pulse before (a, b) and during (c, d) application of 40 μ*M* phloretin. In the untreated fibre, the current with prepulse (b) is smaller than the current without prepulse

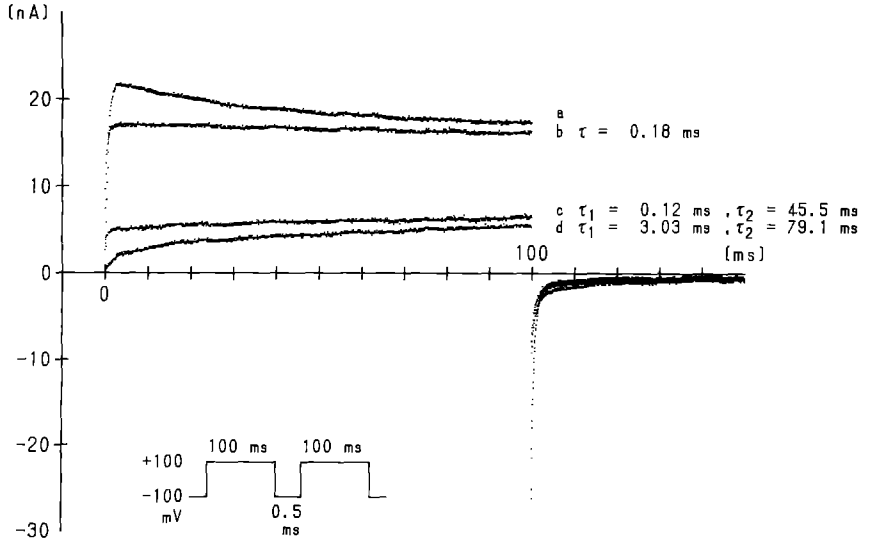


Fig. 8. Effect of a long depolarizing prepulse on the current elicited by a test pulse in control (traces a and b) and in 40 μ*M* phloretin (traces c and d). Inset shows pulse programme. Traces a and d were recorded without prepulse, traces b and c with prepulse. The time constants for the turning-on of the outward current are given. Sensory fibre. Temperature 15 °C

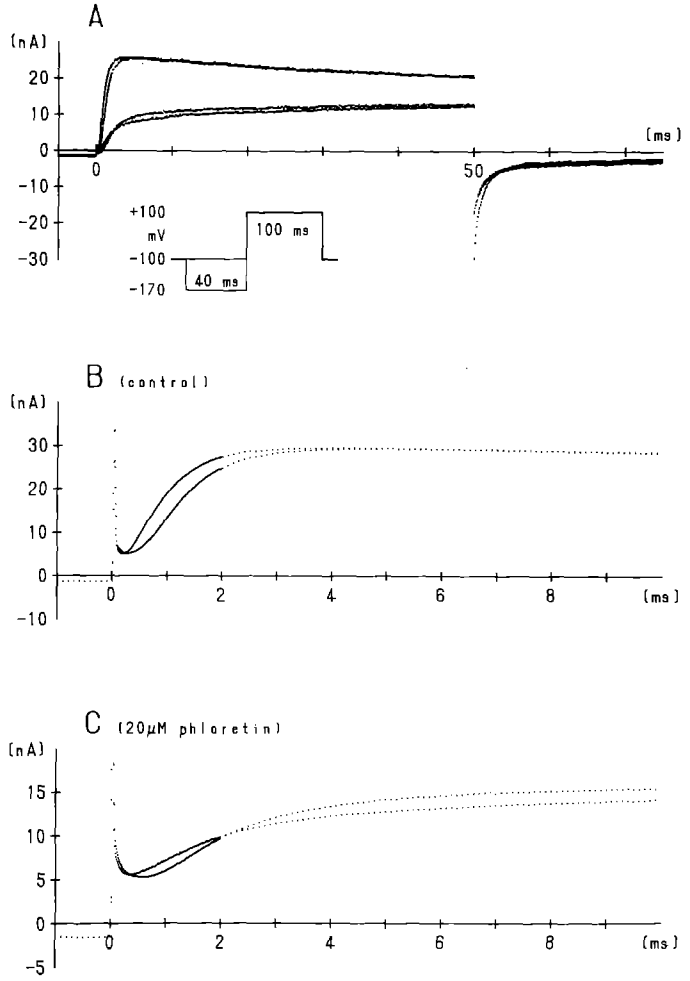


Fig. 9 A–C. Effect of a hyperpolarizing prepulse on the turning-on of I_K. **A** currents in isotonic KCl (larger currents) and in isotonic KCl with 20 μM phloretin (smaller currents) with and without a hyperpolarizing prepulse. Capacitative and leakage current subtracted. In phloretin (but not in control) the current amplitude is slightly increased by the prepulse. **B and C** same records as in **A**, but on expanded time scale and without subtraction of capacitative and leakage current. Both in **B** (control) and in **C** (phloretin) the prepulse delays the turning-on of I_K. Sensory fibre. Temperature 15 °C

(a), probably because of inactivation. During phloretin application the opposite is true: the current with prepulse (c) is larger and rises much faster than the current without prepulse (d). The currents were fitted with one or two exponentials and the time constants (τ or τ_1, τ_2) are given in Fig. 8. In c (phloretin with prepulse) τ_1 is 25 times smaller than in d (phloretin without prepulse) and similar to τ in b (control with prepulse). In a second identical experiment on a motor fibre the prepulse reduced τ_1 in phloretin 12 fold to a value similar to τ_1 in control with prepulse. The observations are reminiscent of similar findings on 4-AP-treated nodes of Ranvier (Ulbricht and Wagner 1976) and suggest removal of phloretin block during depolarization.

In a second series of experiments we studied the effect of a 40 ms hyperpolarizing prepulse on the kinetics of the K outward current. As shown by Palti et al. (1976) and Begenisich (1979), the frog node of Ranvier exhibits the Cole-Moore effect, i.e. the turning-on of I_K is delayed by a preceding hyperpolarization. Figure 9 demonstrates that the Cole-Moore effect is not abolished by phloretin treatment. The delay in turning-on is, however, superimposed by another effect, a slight increase in the current amplitude (which is not seen in untreated fibres).

Discussion

The experiments show that the fast (but not the 4-AP-resistant slow) K channels of the node of Ranvier are affected by phloretin in the same way as the K channels of the squid giant axon (Strichartz et al. 1980; Spires and Begenisich 1989): Blockage of part of the K channels, slowing of the turning-on of the remaining K current, flattening and positive shift of the conductance vs. voltage curve. In squid axons the shift of the conductance curve by 10–50 μM phloretin applied externally is on average 26.7 mV (Table 2 of Strichartz et al., 1980). In our experiments the shift tended to be even larger (e.g. more than 50 mV in Fig. 4 A). There is one important difference between the experiments on squid axons and our experiments: In the former, according to Strichartz et al. (1980), "steady-state effects were reached after 5 min of exposure to phloretin"; in our experiments, the phloretin effect became continually stronger during the first 20 min without showing any clear sign of reaching a steady state. Phloretin is very lipid soluble, having ether-water and octanol-water partition coefficients in excess of 100 (Jennings and Solomon 1976). We think that equilibration with the various lipid structures is a slow process, responsible for the slow development of the phloretin effect. For the same reason the Na current of the squid axon upon exposure to inhalation anaesthetics continues to decline over a period of 10–20 min (Haydon and Urban 1983). It seems surprising that in the phloretin experiments on squid axons 5 min were sufficient to reach a steady state.

In the frog node, phloretin abolishes the distinction between the two types of fast K channels (see Figs. 4 A and 5 A). This could indicate that phloretin has a larger effect on fast 1 than on fast 2. Similarly, increasing the pH shifts the voltage dependence of fast 1 much more strongly than that of fast 2 (Cahalan and Papone 1983). The alternative explanation that phloretin converts fast 1 into fast 2 channels seems less likely because DTX, a selective blocker of fast 1, is effective on nodes pretreated with phloretin.

A simple model which can reproduce most observation on K channel function has been described by Spires and Begenisich (1989). They consider the K channel to be composed of two independent, but not identical, subunits which can exist in two states, labeled 1 and 2 for one subunit and A and B for the other. A channel can conduct only if the subunits are in the 2 and B states, i.e. the fraction of open channels is given by $f_{open} = P_2 \cdot P_B$ where P_2 and P_B are the probabilities of the subunits being in states 2 and B, respectively. With suitably chosen values for the charges q_{12} and q_{AB} moved in the $1 \rightleftharpoons 2$ and $A \rightleftharpoons B$ transitions and for the midpoint potentials V_{12} and V_{AB} of the two charge distributions (see legend of Fig. 10) we obtain a voltage dependence of f_{open} which

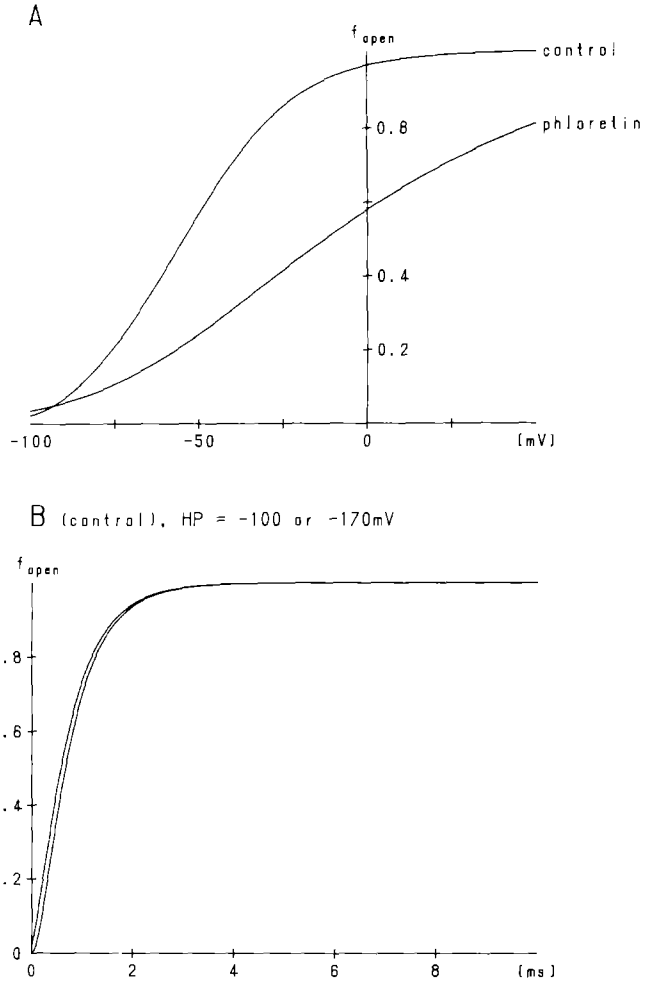


Fig. 10. Predictions of the Spires and Begenisich (1989) model for an untreated and a phloretin-treated Ranvier node. A, fraction of open channels, f_{open}, versus potential in steady-state for control node and for phloretin-treated node. B, time course of f_{open} for a pulse from −100 mV (left curve) or −170 mV (right curve) in control node. C, same for phloretin-treated node. The model assumes two independent subunits which can exist in two conformations: $1 \rightleftharpoons 2$ for one subunit, $A \rightleftharpoons B$ for the other. The fraction of open channels is $f_{open} = P_2 \cdot P_B$. The steady-state values and the time dependence of the probabilities P_2 and P_B were calculated from (6) and (2) of Spires and Begenisich (1989) with the parameters

	control	phloretin
q_{12}	3.0 e	1.0 e
q_{AB}	1.5 e	0.5 e
V_{12}	−95 mV	−65 mV
V_{AB}	−55 mV	−25 mV
τ_{12}	0.4 ms	0.8 ms
τ_{AB}	0.7 ms	1.4 ms

resembles the conductance vs voltage curve of the nodal K channels (Fig. 10A). Figure 6 of Spires and Begenisich (1989) shows that the effect of phloretin on the K channel gating current of the squid axon consists of a marked reduction of q_{12} and q_{AB} and a positive shift of V_{12} and V_{AB}. Accordingly, reducing the charges q_{12} and q_{AB} to a third of the control value and shifting the potentials V_{12} and V_{AB} by 30 mV yields a curve similar to the conductance curves of phloretin-treated nodes (Fig. 10A). As emphasized by Spires and Begenisich (1989), their model reproduces the Cole-Moore effect (Fig. 10B). This type of behavior is also seen in phloretin-treated nodes, both in the experiment (Fig. 9C) and in the model (Fig. 10C). In the model, the slow turning-on of I_K in phloretin has been achieved by doubling the time constants τ_{12} and τ_{AB} (see legend of Fig. 10). We think, however, that the main reason for the slow kinetics of I_K in phloretin-treated nodes is not a slowing of the transitions $1 \rightarrow 2$ and $A \rightarrow B$. Rather, our double pulse experiment (Fig. 8) suggests that the main reason for the slow turning-on of I_K is the depolarization-induced weakening of the phloretin block.

Concerning the question which molecular mechanisms underly the phloretin effect we can add little to the suggestions made by previous authors. As pointed out by Andersen et al. (1976) and Strichartz et al. (1980), field strengths as high as 10^7–10^8 V/cm exist at a distance of 5 Å from a phloretin dipole in low dielectric media, certainly large enough to modify channel gating profoundly. Alternatively, it may be a purely mechanical effect, resulting from the squeezing of phloretin molecules between the phospholipid molecules of the nerve membrane. The depolarization-induced weakening of the phloretin block may be due to potential dependent changes in membrane structure; such changes have been revealed by optical studies (for review, see Keynes 1972). We can offer no explanation for the fact that only the K channels, and only the fast K channels, show the characteristic phloretin effect.

In continuation of the present study we are recording single channel currents from frog nerve fibres with the method of Jonas et al. (1989). We find that the I channels are blocked by 40 μ*M* phloretin whereas the Ca-activated maxi channels (see Jonas et al. 1991) are activated by 5–100 μ*M* phloretin (Koh and Klusemann, unpublished). The latter effect may be responsible for the "supernormal" K outward currents seen in some of our experiments (Fig. 6).

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References

Andersen OS, Finkelstein A, Katz I, Cass A (1976) Effect of phloretin on the permeability of thin lipid membranes. J Gen Physiol 67:749–771

Begenisich T (1979) Conditioning hyperpolarization-induced delays in the potassium channels of myelinated nerve. Biophys J 27:257–266

Benoit E, Dubois J-M (1986) Toxin I from the snake *Dendroaspis polylepis polylepis:* a highly specific blocker of one type of potassium channel in myelinated nerve fiber. Brain Res 377:374–377

Bräu ME, Dreyer F, Jonas P, Repp H, Vogel W (1990) A K^+ channel in *Xenopus* nerve fibres selectively blocked by bee and snake toxins: binding and voltage-clamp experiments. J Physiol 420:365–385

Cahalan MD, Pappone PA (1983) Chemical modification of potassium channel gating in frog myelinated nerve by trinitrobenzene sulphonic acid. J Physiol 342:119–143

Dodge FA, Frankenhaeuser B (1959) Sodium currents in the myelinated nerve fibre of *Xenopus laevis* investigated with the voltage clamp technique. J Physiol 148:188–200

Drouin H, Neumcke B (1974) Specific and unspecific charges at the sodium channels of the nerve membrane. Pflügers Arch 351:207–229

Dubois JM (1981) Evidence for the existence of three types of potassium channels in the frog Ranvier node membrane. J Physiol 318:297–316

Haydon DA, Urban BW (1983) The effects of some inhalation anaesthetics on the sodium current of the squid giant axon. J Physiol 341: 429–439

Hof D (1986) A pulse generating and data recording system based on the microcomputer PDP 11/23. Comput Methods Progr Biomed 23:309–316

Jennings ML, Solomon AK (1976) Interaction between phloretin and red blood cell membrane. J Gen Physiol 67:381–397

Jonas P, Bräu ME, Hermsteiner M, Vogel W (1989) Single-channel recording in myelinated nerve fibers reveals one type of Na channel but different K channels. Proc Natl Acad Sci USA 86:7238–7242

Jonas P, Koh D-S, Kampe K, Hermsteiner M, Vogel W (1991). ATP-sensitive and Ca-activated potassium channels in vertebrate axons: Novel links between metabolism and excitability. Pflügers Arch 418:68–73

Keynes RD (1972) Excitable membranes. Nature 239:29–32

Klusemann J, Meves H (1991) Phloretin, a dipolar organic compound, strongly affects the potassium channels of the frog node of Ranvier. Pflügers Arch 418 [Suppl 1]: R 79

Nonner W (1969) A new voltage clamp method for Ranvier nodes. Pflügers Arch 309:176–192

Palti Y, Ganot G, Stämpfli R (1976) Effect of conditioning potential on potassium current kinetics in the frog node. Biophys J 16:261–273

Pappone PA, Cahalan MD (1984) Chemical modification of potassium channels in myelinated nerve fibers. Treatment with TNBS or high pH causes resistance to block by 4-aminopyridine. Biophys J 45:62–64 (1984)

Plant TD (1986) The effects of rubidium ions on components of the potassium conductance in the frog node of Ranvier. J Physiol 375:81–105

Spires S, Begenisich T (1989) Pharmacological and kinetic analysis of K channel gating currents. J Gen Physiol 93:263–283

Strichartz GR, Oxford GS, Ramon F (1980) Effects of the dipolar form of phloretin on potassium conductance in squid giant axons. Biophys J 31:229–246

Ulbricht W, Wagner H-H (1976) Block of potassium channels of the nodal membrane by 4-aminopyridine and its partial removal on depolarization. Pflügers Arch 367:77–87